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Original article

Regioselective synthetic approaches towards 1,2,8,9-tetraazadispiro[4.1.4.2] trideca-2,9-dien-6-ones of potential antimicrobial properties

Adel S. Girgis [a,*], Flora F. Barsoum [b], Ahmed Samir [c]

[a] Pesticide Chemistry Department, National Research Centre, El-Behoos Street, Dokki, 12622 Cairo, Egypt
[b] Pharmaceutical Chemistry Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt
[c] Microbiology Department, Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt

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ABSTRACT

Reaction of 2,5-bis(arylmethylidene)cyclopentanones **1a–d** with nitrilimines (generated *in situ via* triethylamine dehydrohalogenation of the corresponding hydrazonoyl chlorides **2a,b**) in 1:2 molar ratio proceeds in a high regioselective manner affording monocycloadducts **3** and dicycloadducts in the form of two isomers **4**, **5**. Single crystal X-ray diffraction studies of the isolated crystalline form of **3c** support the established structure and indicate that the formed product is 7*E*, 4*S*, 5*R*. Antimicrobial activity screening of the synthesized compounds **3–5**, utilizing a variety of Gram-positive (*Staphylococcus aureus*, *Enterococcus fecalis* and *Streptococcus agalactiae*), Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae* and *Proteus vulgaris*) and yeast (*Candida albicans*), exhibited that all the prepared analogues acquire promising activities against both Gram-positive and Gram-negative bacteria especially compounds **3b**, **4a** (antimicrobial active agents against Gram-positive bacteria) and **3c** (antimicrobial active agent against Gram-negative bacteria).

1. Introduction

Spiro-compounds have been known for a long time to be present in phytochemicals either in alkaloids, lactones or terpenoids. Many spirocyclic alkaloids were found to be very potent nicotinic receptor antagonists [1]. Spiroketals were reported to be the sub-units of many natural biological interest substances such as insect pheromones, antifeedants and polyether antibiotics [2]. Many spiro-oxindole derivatives were found to possess very wide biological applications as antimicrobial, antitumor and inhibitors of human NK-1 receptor [3–5]. Several azaspiro-compounds were reported to be tachykinin antagonists and of particular use in the treatment of depression, anxiety, pain, inflammation, migraine, emesis or postherpetic neuralgia [6]. The most developed procedure for construction of spiro-containing compounds depends mainly on cycloaddition reactions, especially 1,3-dipolar cycloadditions to exocyclic double bonds [7–9]. Nitrilimines are considered one of the most important dipole systems which used intensively for preparation of diazaspiro-compounds where, great attention was directed towards their reactions due to their high regio- as well as stereoselective properties [10–12].

In the present work, it is intended to investigate the reaction of 2,5-bis(arylmethylidene)cyclopentanones **1** with nitrilimines in an attempt not only to study the regioselectivity of these dipole compounds towards neighbouring olefinic linkages but also to prepare novel dispiro-containing pyrazoline nucleus. The antimicrobial properties of the obtained spiro-containing compounds will be screened utilizing a variety of Gram-positive, Gram-negative bacteria and yeast. The interest in synthesis of pyrazoline containing compounds is attributed to their well known properties as antidepressant [13], monoamine oxidase [14,15] and low density lipoprotein oxidation [16] inhibitory activities in addition to their antimycobacterial "*Mycobacterium tuberculosis*" [17–20] as well as antifungal [21] properties.

2. Results and discussion

2.1. Chemistry

Reaction of 2,5-bis(arylmethylidene)cyclopentanones **1a–d** with nitrilimines (generated *in situ via* triethylamine dehydrohalogenation of the corresponding hydrazonoyl chlorides **2a,b**) in 1:2 molar ratio in refluxing dry benzene afforded monocycloadducts **3** and dicycloadducts **4**, **5**. The structures of the isolated products **3–5** were

* Corresponding author. Tel.: +202 22352405; fax: +202 33370931.
E-mail address: girgisas10@yahoo.com (A.S. Girgis).

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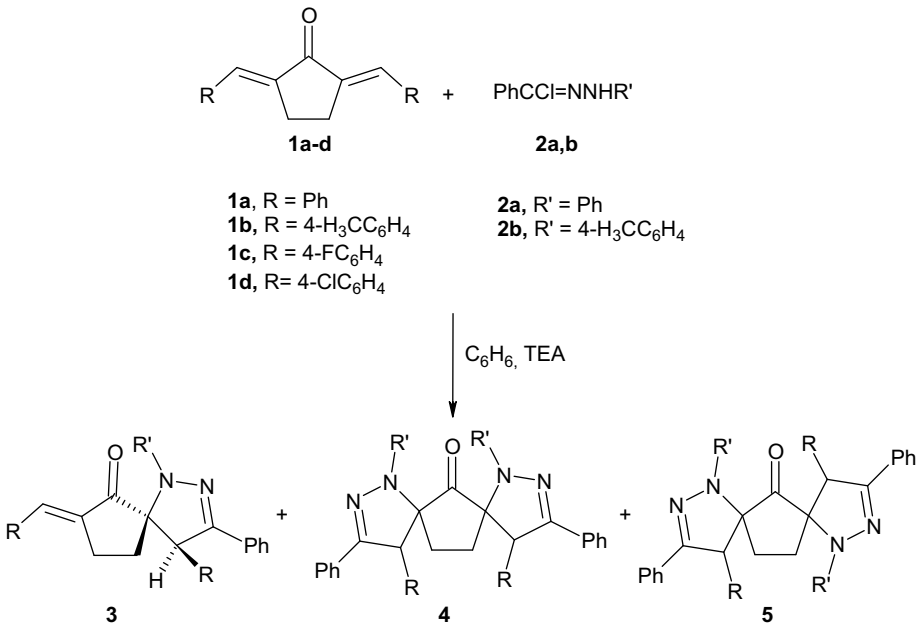

Compd.	R	R'	**3** (mp, °C)	**4** (mp, °C)	**5** (mp, °C)
a	Ph	Ph	53 % (199-201)	19 % (280-282)	---
b	Ph	4-$H_3CC_6H_4$	21 % (234-236)	41 % (287-289)	---
c	4-$H_3CC_6H_4$	Ph	74 % (227-229)	---	---
d	4-FC_6H_4	Ph	16 % (211-213)	29 % (274-276)	---
e	4-FC_6H_4	4-$H_3CC_6H_4$	---	---	45 % (232-234)
f	4-ClC_6H_4	Ph	---	---	56 % (270-272)
g	4-ClC_6H_4	4-$H_3CC_6H_4$	---	---	43 % (260-262)

Scheme 1.

established through different spectroscopic studies (IR, ^{1}H, ^{13}C NMR) as well as elemental analyses data (Scheme 1).

The IR spectra of monocycloadducts **3a–d** reveal the presence of a strong carbonyl stretching vibration band at $\nu = 1712–1707$ cm^{-1} region excluding any cycloaddition reaction pathway that may take place with this moiety. ^{1}H NMR spectra of **3a–d** exhibit the heterocyclic *H*-4 as a sharp singlet signal at $\delta = 4.88–4.92$. The appearance of this signal at the mentioned chemical shift value region is consistent with many similar previously reported structures, supporting the assigned regio-isomeric form [22,23]. In addition, the aliphatic two methylene group protons appear as multiplet signals at $\delta = 1.87–2.93$. However, the olefinic ylidene proton is hidden under the multiplet aromatic protons which makes identification of the geometrical isomerism for the isolated products so difficult based on this spectroscopic technique. Single crystal X-ray diffraction studies of **3c** (Fig. 1) indicated that the isolated product is of 7*E*-configuration. Also, based on these data the established regio-isomeric form structure is completely confirmed and the isolated crystalline form product is inferred to be 4*S*, 5*R*. ^{13}C NMR spectrum (APT) of **3c** reveals the presence of a methine carbon (*C*-4) at $\delta = 61.35$, in addition to a quaternary spiro-carbon (*C*-5) at $\delta = 79.09$. These observed data are in accord with many reported similar structures [22,23] confirming the established regio-isomeric form. From all the above mentioned data, it could be concluded that the attacking dipole compound (nitrilimine) approaches the dipolarophile (enone system, *E*-form configuration) **1** from the less hindered face with retention of the starting geometrically isomeric configuration for the other unreacted double bond residue.

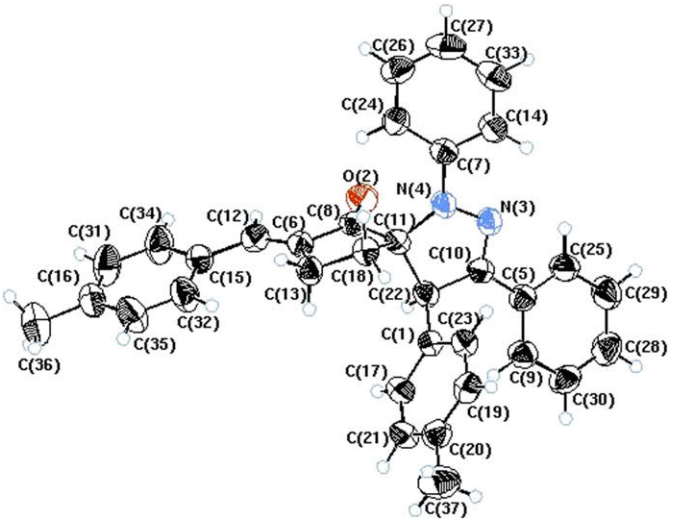

Fig. 1. Single crystal X-ray diffraction of **3c**.

The IR spectra of **4** exhibit the carbonyl band at $\nu = 1751$–$1749\ cm^{-1}$. Meanwhile, ^1H NMR spectra of **4** show a singlet signal integrated for two protons at $\delta = 4.57$–4.61 assignable to the chemically and magnetically equivalent *H*-4 and *H*-11. These chemical shift values are highly related to the observed ones of monocycloadducts **3** confirming the established regio-isomeric form structure. ^{13}C NMR "APT" spectrum of **4b** also adds a conclusive evidence for the assumed structure. Where the pyrazoline HC-4/11 are observed as one signal at $\delta = 60.46$ beside the spiro *C*-5/7 which were exhibited at $\delta = 80.51$. From all the aforementioned data, it could be concluded that the 1,3-dipolar cycloaddition reaction of nitrilimines (dipoles) attack both the two olefinic linkages of dipolarophiles **1** superficially (*i.e.* from the less hindered face as described in the case of monocycloadduct formation **3**) affording eventually **4**.

On the other hand, structure of dicycloadducts **5e–g** was deduced from their IR spectra which reveal a strong carbonyl stretching vibration band at $\nu = 1749$–$1741\ cm^{-1}$. In addition ^1H NMR spectra of **5** exhibit the pyrazoline *H*-4/11 as a singlet signal at $\delta = 3.52$–3.55. The appearance of this signal at the mentioned chemical shift value region is consistent with many similar reported structures, where the dipole molecules attack the olefinic linkages of diene system in a regioselective manner from two opposite faces [24,25]. ^{13}C NMR (APT) spectrum of **5g** adds a good support for the assigned regio-isomeric form structure which reveals the pyrazoline methine carbons HC-4/11 as one signal at $\delta = 56.70$ and the spiro-carbons (*C*-5/7) at $\delta = 80.12$. These observed values are highly related to many previously described similar structures [24,25].

From all the above, it could be concluded that 1,3-dipolar cycloaddition reaction of nitrilimines with 2,5-bis(arylmethylidene)cyclopentanones **1** under the described reaction conditions afforded either monocycloadducts **3** or dicycloadducts **4**, **5** in a high regioselective manner where many efforts have been made for either isolation or identification of any different regio-isomers from the reaction medium but were unsuccessful. The isolation of two dicycloadduct isomeric forms could be attributed to the way of nitrilimine (dipole) attack the diene system at either the same face affording **4** or at different faces giving rise to **5**.

2.2. Antimicrobial activity

Antimicrobial activity screening of the synthesized compounds **3–5** was determined by the agar dilution technique as recommended by the Clinical and Laboratory Standard Institute (CLSI) [26] utilizing a variety of Gram-positive bacteria (*Staphylococcus aureus*, *Enterococcus fecalis* and *Streptococcus agalactiae*), Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae* and *Proteus vulgaris*) and yeast (*Candida albicans*). From the obtained results (Table 1) it has been noticed that, all the tested compounds exhibit promising antimicrobial properties against both Gram-positive and Gram-negative bacteria. However, all the tested compounds seem completely inactive against the used yeast strain (*C. albicans*). It has also generally been noticed that, compounds **3** and **4** are more effective antibacterial agents than compounds **5**. Additionally, compound **3b** seems to be the most effectively prepared Gram-positive antibacterial agent. Otherwise, compound **3c** seems to be the most effectively prepared Gram-negative antibacterial agent. This may be attributed to the role of tolyl function attachment to either 1- or 4-position of pyrazoline ring system as the most observed enhancing antibacterial properties moiety comparable with the other adopted residues. However, this assumed role seems not a general applicable rule governing the observed antibacterial properties concerning tetraazadispiro-ring system, as exhibited in compounds **4a** and **4b**, where the former one "possessing no tolyl residue" is considered more Gram-positive active agent.

Table 1
Minimum inhibitory concentrations (MIC, μg/ml) of the tested compounds against different organisms.

Compounds	3a	3b	3c	3d	4a	4b	4d	5e	5f	5g	CIP*	AMP**
Organisms												
Staphylococcus aureus	5	0.6	2.5	10	1.3	2.5	5	10	10	10	0.3	(−)
Enterococcus fecalis	2.5	1.3	5	5	2.5	5	10	10	10	10	0.3	(−)
Streptococcus agalactiae	2.5	1.3	5	5	2.5	2.5	10	2.5	10	10	0.6	(−)
Escherichia coli	5	5	0.6	10	10	10	5	10	10	10	0.3	(−)
Klebsiella pneumoniae	2.5	2.5	1.3	2.5	10	5	10	10	10	5	1.3	(−)
Proteus vulgaris	2.5	5	1.3	5	10	5	10	10	10	10	0.3	(−)
Candida albicans	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	4

CIP* = Ciprofloxacin; AMP** = Amphotericin B; (−) = inactive.

3. Experimental

Melting points are uncorrected and recorded on a digital Electrothermal 9100 melting point apparatus. IR spectra (KBr) were recorded on a JASCO FT/IR 300E spectrophotometer. ^1H NMR spectra were recorded on Varian GEMINI 200 MHz and Varian MERCURY 300 MHz spectrometers. ^{13}C NMR spectra were recorded on a Varian MERCURY 300 (75 MHz). The starting compounds **1a–d** [27–29] and **2a**,**b** [30,31] were prepared according to the previously reported procedures.

3.1. Synthesis of *3a–d*, *4a*,*b*,*d* and *5e–g* (general procedure)

A mixture of **1a–d** (2.5 mmol) and the corresponding **2a**,**b** (5 mmol) in dry benzene (20 ml) containing triethylamine (7.5 mmol) was boiled under reflux for the appropriate time. The separated triethylammonium chloride was collected and the reaction mixture was evaporated till dryness under reduced pressure. The formed solid upon triturating the residue with methanol (5 ml), was collected and crystallized from a suitable solvent affording the corresponding **3** or **5**. Upon concentrating the solvent of crystallization to the least amount ($\approx$5 ml), **4** was separated. In case of **5e**,**g** the products were purified by silica gel (F_{254}) TLC.

3.1.1. 7-(Phenylmethylidene)-1,3,4-triphenyl-1,2-diazaspiro[4.4]non-2-en-6-one (*3a*)

Reaction time 40 h, orange crystals from n-butanol "through reaction of **1a** and **2a** affording **3a** which was crystallized, filtered and dried, upon concentrating the filterate **4a** was obtained as almost colourless crystals", mp 199–201 °C, yield 53%. IR: ν_{max}/cm^{-1} 1711, 1615, 1595. ^1H NMR ($CDCl_3$): δ 1.89 (dd, $J = 8.4$, 13.8 Hz, 1H, upfield H of CH_2), 2.06–2.25 (m, 1H, downfield H of CH_2), 2.34–2.49 (m, 1H, upfield H of CH_2), 2.79–2.93 (m, 1H, downfield H of CH_2), 4.92 (s, 1H, hetero. *H*-4), 6.90–7.71 (m, 21H, 20 arom. H + olefinic CH). Anal. Calcd. for $C_{32}H_{26}N_2O$ (454.55): C, 84.55; H, 5.77; N, 6.16. Found: C, 84.78; H, 5.96; N, 6.25.

3.1.2. 1,3,4,8,10,11-Hexaphenyl-1,2,8,9-tetraazadispiro[4.1.4.2]trideca-2,9-dien-6-one (*4a*)

Almost colourless crystals from n-butanol "through reaction of **1a** and **2a** affording **3a** which was crystallized, filtered and dried, upon concentrating the filterate **4a** was obtained as almost colourless crystals", mp 280–282 °C, yield 19%. IR: ν_{max}/cm^{-1} 1749, 1593, 1560. ^1H NMR ($CDCl_3$): δ 1.50–1.60 (m, 2H, CH_2), 1.88–1.99 (m, 2H, CH_2), 4.61 (s, 2H, *H*-4/11), 6.90–7.53 (m, 30H, arom. H). Anal. Calcd. for $C_{45}H_{36}N_4O$ (648.77): C, 83.30; H, 5.59; N, 8.64. Found: C, 83.14; H, 5.51; N, 8.48.

*3.1.3. 3,4-Diphenyl-1-(4-methylphenyl)-7-(phenylmethylidene)-1,2-diazaspiro[4.4]non-2-en-6-one (**3b**)*

Reaction time 45 h, orange crystals from ethanol "through reaction of **1a** and **2b** affording **3b** which was crystallized, filtered and dried, upon concentrating the filterate **4b** was obtained as pale yellow crystals", mp 234–236 °C, yield 21%. IR: ν_{max}/cm^{-1} 1707, 1610, 1595. ^{1}H NMR ($CDCl_3$): δ 1.87 (dd, $J = 8.8$ 13.2 Hz, 1H, upfield H of CH_2), 2.10–2.23 (m, 1H, downfield H of CH_2), 2.26 (s, 3H, CH_3), 2.32–2.44 (m, 1H, upfield H of CH_2), 2.75–2.87 (m, 1H, downfield H of CH_2), 4.88 (s, 1H, hetero. *H*-4), 7.02–7.67 (m, 20H, 19 arom. H + olefinic CH). Anal. Calcd. for $C_{33}H_{28}N_2O$ (468.57): C, 84.58; H, 6.02; N, 5.80. Found: C, 84.69; H, 6.14; N, 6.06.

*3.1.4. 1,8-Bis(4-methylphenyl)-3,4,10,11-tetraphenyl-1,2,8,9-tetraazadispiro[4.1.4.2]trideca-2,9-dien-6-one (**4b**)*

Pale yellow crystals from ethanol "through reaction of **1a** and **2b** affording **3b** which was crystallized, filtered and dried, upon concentrating the filterate **4b** was obtained as pale yellow crystals", mp 287–289 °C, yield 41%. IR: ν_{max}/cm^{-1} 1749, 1591, 1560. ^{1}H NMR ($CDCl_3$): δ 1.40–1.53 (m, 2H, CH_2), 1.81–1.90 (m, 2H, CH_2), 2.35 (s, 6H, $2CH_3$), 4.58 (s, 2H, *H*-4/11), 6.80–7.52 (m, 28H, arom. H). ^{13}C NMR "APT" ($CDCl_3$): δ 21.03 (CH_3), 25.80 (CH_2), 60.46 (*C*-4/11), 80.51 (spiro *C*-5/7), 124.55, 126.25, 128.15, 128.19, 128.33, 129.14, 129.33, 129.70 (arom. CH), 131.97, 134.24, 134.72, 141.00, 148.89 (quaternary arom. C), 209.95 (C═O). Anal. Calcd. for $C_{47}H_{40}N_4O$ (676.82): C, 83.40; H, 5.96; N, 8.28. Found: C, 83.15; H, 5.82; N, 8.49.

*3.1.5. 1,3-Diphenyl-4-(4-methylphenyl)-7-[(4-methylphenyl)methylidene]-1,2-diazaspiro[4.4]non-2-en-6-one (**3c**)*

Reaction time 45 h, orange crystals from n-butanol "through reaction of **1b** and **2a** affording **3c**", mp 227–229 °C, yield 74%. IR: ν_{max}/cm^{-1} 1707, 1620, 1599. ^{1}H NMR ($CDCl_3$): δ 1.90 (dd, $J = 8.4$, 13.4 Hz, 1H, upfield H of CH_2), 2.10–2.29 (m, 1H, downfield H of CH_2), 2.34 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 2.38–2.50 (m, 1H, upfield H of CH_2), 2.80–2.93 (m, 1H, downfield H of CH_2), 4.89 (s, 1H, hetero. *H*-4), 6.85–7.69 (m, 19H, 18 arom. H + olefinic CH). ^{13}C NMR "APT" ($CDCl_3$): δ 21.14, 21.51 (CH_3), 24.01, 25.26 (CH_2), 61.35 (HC-4), 79.09 (spiro *C*-5), 116.77, 120.61, 126.52, 127.97, 128.05, 128.77, 129.45, 129.60, 131.11, 136.50 (arom. CH + olefinic CH), 131.78, 132.03, 132.26, 132.34, 137.82, 140.64, 142.58, 147.12 (arom. quaternary C), 204.70 (C═O). Anal. Calcd. for $C_{34}H_{30}N_2O$ (482.60): C, 84.61; H, 6.27; N, 5.81. Found: C, 84.42; H, 6.16; N, 5.88.

*3.1.6. 1,3-Diphenyl-4-(4-fluorophenyl)-7-[(4-fluorophenyl)methylidene]-1,2-diazaspiro[4.4]non-2-en-6-one (**3d**)*

Reaction time 48 h, orange crystals from n-butanol "through reaction of **1c** and **2a** affording **3d** which was crystallized, filtered and dried, upon concentrating the filterate **4d** was obtained as almost colourless crystals", mp 211–213 °C, yield 16%. IR: ν_{max}/cm^{-1} 1712, 1622, 1595. ^{1}H NMR ($CDCl_3$): δ 1.87 (dd, $J = 8.2$, 13.4 Hz, 1H, upfield H of CH_2), 2.04–2.23 (m, 1H, downfield H of CH_2), 2.35–2.51 (m, 1H, upfield H of CH_2), 2.77–2.90 (m, 1H, downfield H of CH_2), 4.90 (s, 1H, hetero. *H*-4), 6.88–7.65 (m, 19H, 18 arom. H + olefinic CH). Anal. Calcd. for $C_{32}H_{24}F_2N_2O$ (490.53): C, 78.35; H, 4.93; N, 5.71. Found: C, 78.49; H, 5.09; N, 5.93.

*3.1.7. 4,11-Bis(4-fluorophenyl)-1,3,8,10-tetraphenyl-1,2,8,9-tetraazadispiro[4.1.4.2]trideca-2,9-dien-6-one (**4d**)*

Almost colourless crystals from n-butanol "through reaction of **1c** and **2a** affording **3d** which was crystallized, filtered and dried, upon concentrating the filterate **4d** was obtained as almost colourless crystals", mp 274–276 °C, yield 29%. IR: ν_{max}/cm^{-1} 1751, 1595, 1560. ^{1}H NMR ($CDCl_3$): δ 1.45–1.56 (m, 2H, CH_2), 1.95–2.05 (m, 2H, CH_2), 4.57 (s, 2H, *H*-4/11), 6.87–7.51 (m, 28H, arom. H). Anal. Calcd. for $C_{45}H_{34}F_2N_4O$ (684.75): C, 78.93; H, 5.00; N, 8.18. Found: C, 79.01; H, 5.11; N, 8.35.

*3.1.8. 4,11-Bis(4-fluorophenyl)-1,8-bis(4-methylphenyl)-3,10-diphenyl-1,2,8,9-tetraazadispiro[4.1.4.2]trideca-2,9-dien-6-one (**5e**)*

Reaction time 50 h, pale yellow crystals "through reaction of **1c** and **2b** affording **5e**" purified by silica gel TLC using chloroform–light petroleum (60–80 °C) mixture as 2:3 v/v for elution, mp 232–234 °C, yield 45%. IR: ν_{max}/cm^{-1} 1749, 1604, 1556. ^{1}H NMR ($CDCl_3$): δ 1.50–1.54 (m, 2H, CH_2), 1.81–1.83 (m, 2H, CH_2), 2.46 (s, 6H, $2CH_3$), 3.55 (s, 2H, *H*-4/11), 6.91–7.32 (m, 26H, arom. H). Anal. Calcd. for $C_{47}H_{38}F_2N_4O$ (712.80): C, 79.19; H, 5.37; N, 7.86. Found: C, 79.35; H, 5.44; N, 7.67.

*3.1.9. 4,11-Bis(4-chlorophenyl)-1,3,8,10-tetraphenyl-1,2,8,9-tetraazadispiro[4.1.4.2]trideca-2,9-dien-6-one (**5f**)*

Reaction time 50 h, yellow crystals from n-butanol "through reaction of **1d** and **2a** affording **5f**", mp 270–272 °C, yield 56%. IR: ν_{max}/cm^{-1} 1741, 1595, 1560. ^{1}H NMR ($CDCl_3$): δ 1.54–1.61 (m, 2H, CH_2), 1.83–1.88 (m, 2H, CH_2), 3.53 (s, 2H, *H*-4/11), 6.84–7.55 (m, 28H, arom. H). Anal. Calcd. for $C_{45}H_{34}Cl_2N_4O$ (717.66): C, 75.31; H, 4.78; N, 7.81. Found: C, 75.40; H, 4.91; N, 8.06.

*3.1.10. 4,11-Bis(4-chlorophenyl)-1,8-bis(4-methylphenyl)-3,10-diphenyl-1,2,8,9-tetraazadispiro[4.1.4.2]trideca-2,9-dien-6-one (**5g**)*

Reaction time 48 h, pale yellow crystals "through reaction of **1d** and **2b** affording **5g**" purified by silica gel TLC using chloroform–light petroleum (60–80 °C) mixture as 2:3 v/v for elution, mp 260–262 °C, yield 43%. IR: ν_{max}/cm^{-1} 1741, 1593, 1560. ^{1}H NMR ($CDCl_3$): δ 1.47–1.50 (m, 2H, CH_2), 1.71–1.79 (m, 2H, CH_2), 2.46 (s, 6H, $2CH_3$), 3.52 (s, 2H, *H*-4/11), 6.80–7.31 (m, 26H, arom. H). ^{13}C NMR "APT" ($CDCl_3$): δ 20.95 (CH_3), 25.94 (CH_2), 56.70 (HC-4/11), 80.12 (spiro *C*-5/7), 125.99, 126.13, 128.14, 128.56, 129.10, 129.52, 130.23 (arom. CH), 131.23, 133.13, 133.81, 135.68, 142.03, 151.39 (quaternary arom. C), 208.83 (C═O). Anal. Calcd. for $C_{47}H_{38}Cl_2N_4O$ (745.71): C, 75.70; H, 5.14; N, 7.51. Found: C, 75.49; H, 5.04; N, 7.34.

*3.2. Single crystal X-ray crystallographic data of **3c***

Compound **3c** was recrystallized as prismatic orange crystals from n-butanol. The crystallographic data were collected at $T = 298$ K on a Kappa CCD Enraf Nonius FR 590 diffractometer using a graphite monochromator with Mo-K_α radiation ($\lambda = 0.71073$ Å). The crystal structure was determined by SIR97 [32] and refined by maXus [33] (Bruker Nonius, Delft and MacScience, Japan). Chemical formula $C_{34}H_{30}N_2O$, $M_r = 482.627$, triclinic, crystallizes in space group P-1, Cell lengths "$a = 10.4100(3)$, $b = 11.0315(4)$, $c = 12.4118(5)$ Å", Cell angles "$\alpha = 101.044(2)$, $\beta = 104.167(2)$, $\gamma = 101.047(2)°$", $V = 1313.11(8)$ Å^3, $Z = 4$, $D_c = 2.441$ mg/m^3, θ values 2.910–27.485°, absorption coefficient μ (Mo-K_α) = 0.15 mm^{-1}, $F(000) = 1024$. The unique reflections measured 9858 of which 2862 reflections with threshold expression $I > 3\sigma(I)$ were used in the structural analysis. Convergence for 334 variable parameters by least-squares refinement on F^2 with $w = 1/[\sigma^2(F_o^2) + 0.10000F_o^2]$. The final agreement factors were $R = 0.048$ and $wR = 0.083$ with a goodness-of-fit of 1.391. Full crystallographic details excluding structure factors; have been deposited at Cambridge Crystallographic Data Centre (CCDC) as supplementary publication number CCDC 713181.

3.3. Antimicrobial activity screening

Antimicrobial activity screening of the synthesized compounds **3**–**5** was determined by the agar dilution technique as recommended by the Clinical and Laboratory Standard Institute (CLSI) [26]. The tested compounds were dissolved in dimethyl sulfoxide

(DMSO). An inoculum of about 1.5×10^8 colony forming unit (CFU) per spot was applied to the surfaces of Mueller–Hinton agar plates containing graded concentrations of the respective compound; plates were incubated at 37 °C for 18 h. The spot with the lowest concentration of compound showing no growth was defined as the minimum inhibitory concentration (MIC). All organisms used in this study were standard strains obtained from American Type Culture Collection (ATCC). The organisms included representatives of Gram-positive bacteria (*S. aureus* 25923, *E. fecalis* 29212 and *S. agalactiae* 123860), Gram-negative bacteria (*E. coli* 25922, *K. pneumoniae* 33495 and *P. vulgaris* 13315) and yeast (*C. albicans* 20260). The MIC of Ciprofloxacin and Amphotericin B was determined concurrently as reference for antibacterial and antifungal activities, respectively (Table 1). Control DMSO was carried out with each experiment.

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